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Is hyperbaric oxygen or ozone effective in experimental endocarditis?

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ABSTRACT

Background: Infective endocarditis, a disease with high mortality and morbidity, is most commonly caused by Staphylococcus aureus; mortality and morbidity further increase in the presence of methicillin-resistant strains of S. aureus. Linezolid is the first of the oxazolidinones, a new antibiotic group that has been approved for the treatment of infections caused by gram-positive cocci. Linezolid reduces the quantity of microorganisms in vegetation to some extent; in addition, the use of hyperbaric oxygen (HBO) and ozone (O_3) therapies is likely to improve targeted antibacterial effect.

Materials and methods: Fifty-six adult male Wistar rats weighing 300–350 g were used. The subjects were divided into groups as follows: Group 1 ($n = 8$): control group that was not inoculated with microorganisms and was untreated; Group 2 ($n = 8$): control group that was inoculated with microorganisms but was untreated; Group 3 ($n = 8$): linezolid treatment group; Group 4 ($n = 8$): O_3 therapy group; Group 5 ($n = 8$): HBO therapy group; Group 6 ($n = 8$): linezolid + O_3 therapy group; Group 7 ($n = 8$): linezolid + HBO therapy group.

Results: In terms of reducing the number of colonies in the aortic valve, linezolid + HBO therapy was found to be the most effective treatment. Then, respectively linezolid + O_3, linezolid, HBO, and O_3 were found to be effective.

Conclusions: We found that linezolid significantly reduced the number of bacteria in the vegetation in the experimental endocarditis model, and HBO therapy increases the effectiveness of linezolid and makes this better than O_3.

1. Introduction

Infective endocarditis, a disease with high mortality and morbidity, is most commonly caused by *Staphylococcus aureus*; mortality and morbidity further increase in the presence of methicillin-resistant strains of *S aureus* (MRSA). The standard treatment for endocarditis is vancomycin; however, resistance rates have been gradually increasing in recent years [1–5], and the risk of adverse effects of the drug is extremely high. Linezolid is one of the extremely limited good alternatives to vancomycin. Linezolid is the first of the oxazolidinones, a new antibiotic group that has been approved for the

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treatment of infections caused by gram-positive cocci. In our earlier study on an experimental endocarditis model, linezolid significantly reduced the number of bacteria in vegetations, but, it did not provide complete sterilization [6]. This suggests the need for adjuvant therapy. Linezolid reduces the number of colonies of microorganisms in vegetations to some extent [7], but the additional use of hyperbaric oxygen (HBO) and ozone (O_3) therapies is likely to improve the targeted antibacterial effect.

HBO therapy can be simply described as breathing 100% oxygen at pressures greater than 1 atm in a totally closed chamber [8]. It is synergistic and additive to the effects of some antimicrobial agents [9]. The efficacy of HBO therapy for experimental mediastinitis and endocarditis has been reported [10,11].

O_3 is an unstable molecule consisting of three oxygen (O) atoms [12]; it is also a strongly oxidizing gas with antibacterial properties, which provide additional support for different antibiotics [13,14]. In the health sector, O_3 therapy is based on the application of an O_2/O_3 mixture in an amount that does not exceed the antioxidant capacity of biological systems.

This study aimed to investigate whether HBO or O_3 therapy increases the efficacy of linezolid in the treatment of experimental endocarditis. This study is expected to contribute to the treatment of endocarditis.

2. Materials and methods

The methods used for animal experiments were in accordance with the international guiding principles for biomedical research involving animals recommended by the World Health Organization. Permission was granted by Canakkale Onsekiz Mart University Animal Experiments Local Ethics Committee (protocol number: 2013/05-05, date of approval: May 30, 2013). This study was conducted in Canakkale Onsekiz Mart University Experimental Research Center.

In the study, 56 adult male Wistar rats weighing 300–350 g were used. We determined the minimum number of rats so as to accurately reflect the study results and provided normal food and water to the rats *ad libitum*.

The rats were anesthetized by the intramuscular administration of a 2:1 mixture of 0.75 mg/kg of ketamine hydrochloride (100 mg/mL) and xylazine hydrochloride (20 mg/mL). A polyethylene catheter was passed from the right carotid artery to the left ventricle to injure the aortic valve. Insertion of the catheter caused aortic lesions and induced bacteremia, which led to the development of aortic valve vegetations [15,16]. After 18–24 h, all animals, except those in the non-contaminated control group, were intravenously administered 10^6 colony-forming units (CFU) of MRSA in 1 mL of 0.9% NaCl. The catheters were left in place during the entire study. The rats in the untreated groups (contaminated and non-contaminated groups) were killed 48 h after bacterial inoculation to determine the microbial load. Treatment was started 48 h after inoculation and lasted for 5 d. The rats in the treatment groups were killed 12 h after the last dose.

The subjects were divided into groups as follows:

Group 1 ($n = 8$): control group that was not inoculated with microorganisms and was untreated.

Group 2 ($n = 8$): control group that was inoculated with microorganisms but was untreated.

Group 3 ($n = 8$): linezolid treatment group.

Group 4 ($n = 8$): O_3 therapy group.

Group 5 ($n = 8$): HBO therapy group.

Group 6 ($n = 8$): linezolid + O_3 therapy group.

Group 7 ($n = 8$): linezolid + HBO therapy group.

The treatment protocols are summarized in Table 1.

2.1. Assessment of infection

After the extracted vegetations were weighed and titrated in sterile tubes, serial dilutions were performed by homogenizing the vegetations in 1 mL of sterile phosphate saline buffer. These solutions were plated on blood agar plates with 7% sheep blood. All plates were incubated for 48 h at 37°C. Isolated colonies were evaluated for MRSA. The number of bacteria was determined and calculated for each plate in terms of CFU/g tissue.

3. Statistics

Quantitative variables were presented as arithmetic mean ± standard deviation of log10 CFU/g of vegetation. Differences among the groups were investigated using the Student *t* or Mann–Whitney *U* test for continuous variables. Kruskal–Wallis testing was used for comparison between the groups. A P value < 0.05 was considered statistically significant. All statistical studies were carried out with the Statistical Package for the Social Sciences (SPSS) program (version 15.0, SPSS, Chicago, IL).

4. Results

No sign of infection was observed on the valves of the rats in the non-contaminated group (Group 1); there was also no bacterial growth in cultures. Purulence was observed on the valve leaflets of the rats in the contaminated group. The distribution of CFU values according to the groups is summarized

Table 1 – Treatment protocols.

Agent	Route of administration
Linezolid	Intravenously, 75 mg/kg/8 h on the first day; then 75 mg/kg/12 h. Treatment was continued for 5 d.
Ozone	O_3/O_2 gas mixture was given intraperitoneally 0.7 mg/kg. O_3 production was performed with an ozone generator, which is in commercial use. O_3/O_2 (97% O_2, 3% O_2) flow speed was kept constant at 3 L/min, at a concentration of 60 mg/mL. Tygon polymer tubes and silicone-coated polypropylene disposable syringes were used for continuous administration of O_3 at the desired concentration. Treatment was continued for 5 d.
Hyperbaric oxygen	Hyperbaric oxygen therapy at 2.8 atmospheric pressure was given to this group of rats for 90 min every day. Treatment was continued for 5 d.